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Original contribution

Metastatic ocular melanoma to the liver exhibits infiltrative and nodular growth patterns☆

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Summary We examined liver specimens from 15 patients with uveal melanoma (UM) who had died of their disseminated disease. We found 2 distinct growth patterns of UM metastasis: infiltrative (n = 12) and nodular (n = 3). In the infiltrative pattern, individual UM cells with a CD133+ cancer stem cell–like phenotype were present and formed aggregates of stage I <50-μm-diameter micrometastases in the sinusoidal spaces. These micrometastases appeared to expand, destroy adjacent hepatocytes, and form stage II 51- to 500-μm-diameter and then stage III >500μm-diameter metastases, which were encapsulated by collagenized fibrous septae. In the nodular growth pattern, CD133+ melanoma cells aggregated adjacent to portal venules and subsequently appeared to grow and efface the adjacent hepatocytes to form stage II 51- to 500-μm-diameter nodules that surrounded the portal venule. These avascular nodules appeared to further expand to form stage III >500-μm-diameter nodules that exhibited vascularization with minimal fibrosis. The tumor stem cell–like phenotype seen in individual UM cells was lost as the tumors progressed. There were CD56+ natural killer cells in sinusoidal spaces and CD3+ lymphocytes in periportal areas. The nodular growth pattern showed UM cells expressing MMP9 and VEGF. UM cells in both above-described growth patterns exhibited variable BAP1 expression. We propose that changes in the liver microenvironment are related to metastatic UM growth. We hypothesize that these changes include immune regulation within the sinusoidal space for the infiltrative pattern and changes in the VEGF/PEDF ratio for the nodular pattern.

1. Introduction

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults with an annual incidence of 6 to 7 cases/1 000 000 in the white population [1]. Approximately

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40% of UM metastasizes to the liver within 10 years of diagnosis of the primary intraocular tumor [2], and when UM metastasizes, the liver is typically the initial site of metastasis in 95% of cases [3]. The life expectancy of patients with metastatic UM is only 4 to 6 months [1], and at present there are no highly effective treatments for these metastases [4].

The Zimmerman hypothesis, which was formulated in the late 1970s, proposed that enucleation of the eye for UM potentiated hematogenous spread of the tumor and a 2-year postenucleated risk of death [5]. This theory was subsequently disproven, particularly when investigators calculated primary and metastastic UM doubling times. Their extrapolation demonstrated that small, clinically undetectable UM micrometastases were present in the liver, even at the time of diagnosis of the primary intraocular tumor in patients who ultimately died of hepatic metastasis [6,7]. Our laboratory corroborated those results when we found small hepatic UM micrometastases in liver specimens obtained postmortem from patients who died from metastastic UM [8].

In our prior study [8], we found three stages of metastases: stage I: <50-μm-diameter micrometastases within the sinusoidal space; stage II: 51- to 500-μm-diameter metastases that formed expanded collections of cells within the sinusoidal space; and stage III: >500-μm-diameter collections of cells that formed 2 patterns. We characterized the 2 stage III patterns as the "lobular" and "portal" patterns [8]. The lobular pattern appeared to be an expansion of stage I to III intrasinusoidal growth, which infiltrated the portal lobule and was surrounded by fibrous septae. In contrast, the portal pattern appeared to efface, rather than infiltrate, the surrounding liver; we hypothesized that the portal pattern arose in the perivascular area of the portal triad [8].

In this study, we examined liver specimens from an additional 15 patients with UM who had died from metastatic disease. We examined for the growth stages and growth patterns as previously described [8] and explored mechanisms that may account for these growth patterns. Based on our findings, we propose the term "infiltrative" for the lobular pattern and "nodular" for the portal pattern.

2. Materials and methods

This study was approved by the Emory University School of Medicine Institutional Review Board, Atlanta, GA. Sections of livers obtained postmortem from patients who had UM were obtained from the Department of Molecular and Clinical Cancer Medicine, Royal Liverpool and Broadgreen University, Liverpool, UK (C.M. and S.E.C.) and the Department of Ophthalmology, University of Copenhagen, Copenhagen, Denmark (S.H.). The patients' samples had been pseudoanonymized for data other than age, sex, and cause of death. For each case, sections of the livers including an area of suspected metastasis found during autopsy were obtained. The sections were fixed in 10% neutral buffered formalin, routinely processed, and 4-μm sections were obtained by a microtome and placed on slides. The sections measured from 2.0 × 0.9 cm to 3.4 × 2.6 cm. Sections from each case were stained with hematoxylin and eosin (H&E) and Masson trichrome.

Immunohistochemical (IHC) staining using the avidin-biotin complex technique (Dako, Carpinteria, CA) was used to stain other sections from each case for HMB45 (1:50), BAP1 (C-4, Santa Cruz, Insight Biotechnology Ltd, Middlesex, UK, 1 $\mu g/ml^{-1}$), CD3 (Leica Microsystems, Bannockburn, IL, prediluted), CD20 (Leica Microsystems, prediluted), and CD31 (Dako, 1:180). A red chromogen (Vector Red) was used for HMB45 and BAP1, and a brown chromogen (3,3′-diaminobenzidine) was used for CD3, CD20, and CD31. For immunofluorescence (IF) staining, sections of the livers were blocked in normal goat serum (5%) in phosphate-buffered saline, followed by incubation with primary antibody. After washing, sections were incubated with appropriate fluorescent-conjugated secondary antibody, counterstained with 4′,6-diamidino-2-phenylindole (DAPI, Vector Laboratories), and mounted (Vector Laboratories). IF staining for each case was performed for CD56 (R&D Systems, Minneapolis, MN), CD3 (Abcam, Cambridge, MA), CD133 (Biorbyt, San Francisco, CA), HMB45 (ABCAM, Cambridge, MA), MMP9 (Cell Signaling Technology, Boston, MA), VEGF (AMCAM), and a dual-labeled HMB45/CD133. IF was examined by confocal microscopy (Nikon CI R1, Tokyo, Japan). The H&E, Masson trichrome, and IHC-stained slides were examined by light microscopy to determine the presence and distribution of stage I, stage II, and stage III metastases as previously described [8]. The number of these 3 stages of metastases was determined

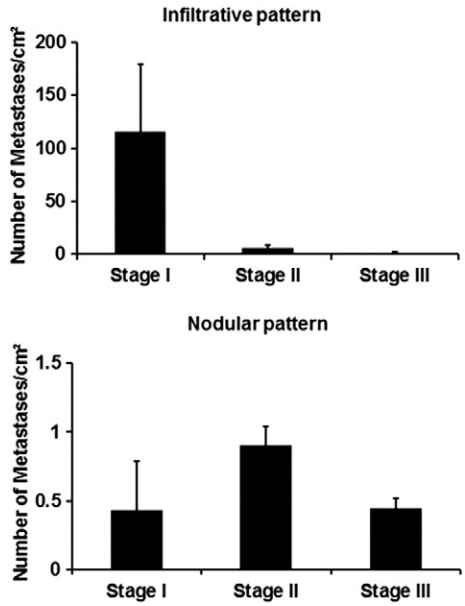

Fig. 1 Number of metastases per area of stage I, II, and III infiltrative and nodular metastases. In the infiltrative pattern, there was a significant decrease in numbers going from stage I to stage III metastases. In the nodular growth pattern, there was approximately 1 metastasis per 2 cm^2 for all 3 stages. SDs are shown.